Differential sensitivity of ammonia oxidising archaea and bacteria to matric and osmotic potential

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Abstract

Microbial oxidation of ammonia controls the rates of nitrification in the majority of soils. Both nitrification rate and the composition of communities of ammonia oxidising archaea (AOA) and ammonia oxidising bacteria (AOB) are influenced by drought, with evidence that AOA are more sensitive to periods of drought than AOB. This has been explained by greater sensitivity of AOA to ammonia concentration, which will increase in soil solution during drought, but an alternative, previously unexplored explanation, is greater sensitivity of AOA to matric and/or osmotic stress. A soil microcosm experiment was designed to distinguish these different explanations in which AOA and AOB abundances (*amoA* abundance) and nitrification rate were measured over 28 days in nine treatments corresponding to all combinations of three soil matric potentials and three initial ammonia concentrations. Comparison of *amoA* abundance dynamics suggested that AOA were more susceptible to reduced matric potential than AOB, irrespective of soil ammonia concentration. The greater sensitivity of soil AOA to osmotic stress was also tested in 10-day cultures of representative strains of AOA and AOB in liquid medium containing different concentrations of NaCl and sorbitol as osmo-inducer. AOA were significantly more sensitive to osmotic stress than AOB. These results provide evidence for greater sensitivity of AOA than AOB to both components of water stress, matric and osmotic potential, representing an additional niche differentiation between these two essential groups of ammonia oxidisers.

1. Introduction

The frequency of drought events, including those in previously temperate regions, is predicted to increase dramatically during the next few decades (Kovats et al., 2014). Reduction in soil water content during drought decreases the mobility and availability of soluble and diffusible substrates and products, increases diffusion of gaseous compounds, including oxygen, and increases water stress. Water stress can arise from both matric stress of cells, for example through increased surface tension caused by desiccation, and osmotic stress of cells in the soil solution, through reduced water activity (Potts, 1994). Sensitivity to disturbances, including water stress, differs between microbial groups with different physiological characteristics and these differences, coupled with changes in the distribution of essential substrates, influence the abundance and activity of soil microbial functional groups (Schimel et al., 2007). Understanding the differential response of microbial groups to drought, especially functional groups involved in crucial biogeochemical cycles, is critical for prediction and mitigation of the impacts of climate change.

Both ammonia oxidising archaea (AOA) and bacteria (AOB) perform the first step in soil nitrification, the oxidation of ammonia (NH_3), via nitrite (NO_2^-), to nitrate (NO_3^-) (Prosser, 2011; Nicol et al., 2011). Nitrification significantly reduces nitrogen fertiliser use efficiency, causes significant pollution of waters through NO_3^- leaching (Puckett et al., 1999), and provides nitrate for denitrification and resultant nitrous and nitric oxide production (Butterbach-Bahl et al., 2013). Drought may alter NH_3 oxidiser (AO) activity through effects of water stress on archaeal and bacterial cells and modification of both the concentration and availability of NH_3 and ammonium (NH_4^+), its protonated form, which is largely dominant in acidic to neutral pH soils. Drying of soil pores through evapotranspiration reduces the number of anaerobic microsites and increases bulk NH_4^+ concentration (due to a reduced volume of water), which may favour NH_3 oxidation. However, soil drying also reduces the thickness of water films on the surface of soil particles, thereby decreasing the movement and availability of NH_4^+ and

NH_3. Drought has been shown to decrease general nitrification activity in soil (Stark and Firestone, 1995), but may have differential effects on members of the AO community. For example, there is evidence that AOA are more sensitive to drought than AOB and less resilient following rewetting of a non-drought acclimated soil (Thion and Prosser, 2014) and this was proposed to result from increases in bulk NH_4^+ concentration. There is indeed evidence of niche differentiation between AO associated with concentration and supply of NH_4^+ and observations that AOB may be favoured in heavily fertilised soils (Di et al., 2010; Verhamme et al., 2011), while AOA predominate in soils receiving low rates of NH_4^+ supply, e.g. through mineralisation of organic nitrogen (Levičnik-Höfferle et al., 2012; Stopnišek et al., 2010). These preferences have been suggested to result from greater ammonia affinity and greater sensitivity to high NH_3 concentration in AOA, although recent studies challenge these proposals (Hink et al., 2017a; Kits et al., 2017; Lehtovirta-Morley et al., 2016). Reduction in NH_4^+ transport during drought may therefore lead to high localised NH_4^+ concentration, potentially benefiting AOB. In support of this, Gleeson et al. (2010) observed an increase in AOB, but not AOA abundance as soil water-filled pore space (WFPS) decreased. In contrast, Vasileiadis et al. (2012) reported a reduction in the abundance of transcripts of AOB *amoA* (encoding ammonia monooxygenase, catalysing NH_3 oxidation), but not those of AOA, when soil moisture content decreased from 87 to 50% water holding capacity (WHC). Differential effects of drought on AOA and AOB may also reflect differences in physiological response to water stress. With the exception of extreme halophiles, both archaea and bacteria respond to osmotic stress by accumulating compatible solutes, protecting cells against osmotic stress, although the chemical nature of those compounds differs (Roeßler and Müller, 2001). Thus, despite domain-level differences in these compounds (Roeßler and Müller, 2001), membrane lipid composition (Elling et al., 2017) and transcriptional machinery, there is no evidence of general differences in sensitivity of AOA and AOB to water stress, although no comparative physiological studies have yet been performed.

AOA and AOB differ in their environmental impact through different ecophysiological characteristics, including cell specific rates of ammonia oxidation (Prosser and Nicol, 2012) and nitrous oxide emissions from soil (Hink et al., 2017b, 2018). Their response to drought may be important in anticipating the effects of increased frequency of drought events on AO community structure and activity and on ecosystem functions relying on nitrification. The aim of this study was to assess the different effects of water stress and NH_4^+ concentration on AO growth and activity, hypothesising that AOB are less sensitive to drought than AOA, because of greater preference for high NH_4^+ concentration during drought, rather than because of different response to water stress. To test this hypothesis, nitrification activity and changes in AOA and AOB abundances were determined in soil microcosms in which NH_4^+ concentration and matric potential were manipulated. To explore AO differential sensitivity to water stress further, the effect of osmotic stress on growth of cultivated AOA and AOB was assessed in laboratory culture.

2. Materials and methods

2.1. Microcosm construction and incubation

Microcosms were constructed using a non-drought-acclimatised agricultural soil (0 – 15 cm depth, pH 6.5) collected from field plots at SRUC, Craibstone, Scotland (grid reference NJ872104). Details of the sampling site and other soil characteristics are described by Kemp et al. (1992). Soil was air-dried at 25°C for 4 days, sieved (3.35-mm mesh) and stored at 4°C for 4 weeks. Soil pH and moisture content were determined as described by Nicol et al. (2005) and initial soil moisture content decreased from 27.7% (field moisture content) to 10% after air-drying. A soil water retention curve, assessing the relationship between soil matric potential and soil moisture, was determined on independent soil samples, measuring matric potential using a WP4C water potentiometer (Decagon, Pullman, UK) (data not shown). Soil microcosms were established in sterile 100-ml Duran glass bottles containing 10 g equivalent dry soil and sufficient sterile distilled water to achieve an initial moisture content of 30% (g

121 water g^{-1} dry soil), corresponding to a matric potential of -0.019 MPa (equivalent to field
122 conditions). Microcosms were pre-treated in two consecutive 9-day incubation cycles to
123 oxidise all ammonia released through mineralisation after wetting the soil. In each cycle,
124 microcosms were incubated in the dark at 30 °C and 30% moisture content for 5 days,
125 maintaining aerobic conditions by removing plastic screw caps for 5 - 10 min every third day
126 and replacing water lost through evaporation by addition of sterile distilled water. Screw caps
127 were then replaced with sterile cotton wool plugs and microcosms were incubated for a further
128 4-day period, during which moisture content decreased to ~12%.

129 After pre-treatment, nine treatments were applied in a full factorial design consisting of
130 all combinations of three NH_4^+-N concentrations and three matric potentials. Moisture content
131 was adjusted to 16.5, 20 or 30% with sterile distilled water to achieve low (-0.080 MPa),
132 medium (-0.051 MPa) and high (-0.019 MPa) matric potentials, respectively. (Matric potential
133 represents the negative pressure applied to remove water from soil. Increasingly negative
134 values of matric potential (lower values) therefore reflect increasing matric stress). Microcosms
135 were also amended with ammonium sulphate ($(NH_4)_2SO_4$) solution to give soil solution
136 concentrations of 0.6, 6 and 60 μg NH_4^+-N g^{-1} soil, termed low, medium and high NH_4^+-N,
137 respectively. Sealed microcosms were incubated in the dark at 30 °C for 28 days, maintaining
138 aerobic conditions as above. Triplicate microcosms for each treatment were destructively
139 sampled after incubation for 0, 7, 14, 21 and 28 days. For each microcosm, 2 g soil was stored
140 at -80 °C for molecular analysis, the remainder being stored at -20 °C for analysis of pH, NH_4^+
141 and NO_x ($NO_3^- + NO_2^-$) concentrations.

142 *2.2. Growth of ammonia oxidisers in liquid medium*

143 The effect of osmotic potential on cultivated AOA and AOB was assessed during batch
144 growth in liquid medium containing different concentrations of NaCl or sorbitol and inoculated
145 with a pure culture of either the AOA, *Candidatus* Nitrosotalea sinensis Nd2 or *Candidatus*

Nitrosocosmicus franklandus C13, or the AOB, *Nitrosomonas europaea* (ATCC 19718) and *Nitrosospira multiformis* (ATCC 25196). *Ca.* N. sinensis was grown in freshwater medium (FWM) at pH 5.0 as described by Lehtovirta-Morley et al. (2011) buffered by adding 2.5 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH 5.35) and 4 mM $NaHCO_3$. *Ca.* N. franklandus was grown in FWM adjusted to pH 7.5 as described by Lehtovirta-Morley et al. (2016). *N. europaea* and *N. multiformis* were cultured in Skinner and Walker medium (Skinner and Walker, 1961) adjusted to pH 7.9. NaCl, routinely used to investigate osmotic stress in heterotrophic bacteria (e.g. Humphrey, 2004), was first chosen as a model osmo-inducer. Sorbitol, a non-toxic and non-electrolyte osmolyte (Suga et al., 2003), was then used to distinguish effects of osmotic stress and NaCl, as Na^+ may have cytotoxic effects on microbial cells (Lanyi, 1979). Growth media were adjusted to seven osmotic potentials (ψ) by addition of NaCl or sorbitol at concentrations of 0 (control), 0.05, 0.1, 0.2, 0.25, 0.3 and 0.4 M. The relationship between osmotic potential and NaCl and sorbitol concentrations used is shown in Table S1. Triplicate batch cultures were grown in 100-ml Duran bottles containing 50 ml FWM and inoculated with 2% (v v^{-1}) of exponentially growing cells of each AOA and AOB. AOA and AOB cultures were incubated in the dark without shaking at 35ºC and 28ºC, respectively, and growth medium (100 µl) was sampled daily during incubation for 10 days for colorimetric assay of nitrite (NO_2^-) concentration. Potential contamination by heterotrophs was assessed by plating on 5% nutrient agar medium, incubated for 10 days at the same temperature as the liquid cultures.

2.3. Chemical analysis

NH_4^+ and NO_x (NO_3^- and NO_2^-) concentrations in soil were determined by colorimetric analysis. NH_4^+-N and NO_x-N were extracted from 2 g soil with 10 ml 1 M KCl and centrifuged at 3,000 rpm for 15 minutes. Concentrations in supernatants were measured as described by Catão et al. (2016). NO_2^- concentration in soil extracts was negligible and NO_x-N concentration is referred to as NO_3^- concentration. NO_2^--N concentration was assessed in liquid cultures as

described by Lehtovirta-Morley et al. (2011) and maximum specific growth rate in batch culture was estimated as the gradient of independent semi-logarithmic plots of NO_2^- concentration *vs*. time during exponential growth, as described by Powell and Prosser (1992).

2.4. Quantification of amoA *genes*

DNA was extracted from 0.5 g of soil according to Griffiths et al. (2000), with modifications (Nicol et al., 2005), and DNA concentration and purity were measured using a Nanodrop ND-2000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Abundances of AOA and AOB in soil microcosms were estimated by quantitative PCR (qPCR) amplification of *amoA* genes using the primers CrenamoA23F/CrenamoA616R (Tourna et al., 2008) and amoA-1F/amoA-2R (Rotthauwe et al., 1997), respectively, with 5 µl of 2 ng μl^{-1} DNA template in 20 µl final volume reactions. AOA and AOB *amoA* standards were prepared using *Ca.* N. sinensis and *N. multiformis*, respectively, as described in Thion and Prosser (2014). qPCR assays were performed on a Master cycler® realplex^2 thermocycler (Eppendorf, Germany) with QuantiFast^{TM} qPCR master mix (Qiagen, Crawley, UK) as described in Thion and Prosser (2014). Amplicon size was verified on a 1% agarose gel electrophoresis and qPCR efficiencies for amplification of AOA and AOB *amoA* genes were 88 - 95% and 90 - 92%, respectively, with r^2 values >0.99.

2.5. Statistical analysis

All statistical analyses were computed using the program R 3.2.2 (R Development Core Team, 2015), using *agricolae* and *nlstools* packages. AOA and AOB abundances were normalised by $\log_{10}$ transformation and mean z-score-transformed. For the soil microcosm experiment, the effects of matric potential, NH_4^+ amendment and time, and their interactions, on soil pH, NH_4^+-N and NO_3^--N concentrations and AOA and AOB *amoA* abundances during incubation of soil microcosms were analysed using three-way ANOVAs. (All independent variables were categorical and destructive sampling allowed analysis of time as an independent

fixed factor.) Generalised linear models were used to explore further the correlations between AOA and AOB abundances, as dependent variables, and matric potential and time as categorical independent variables and measured soil NH_4^+-N concentrations and pH as continuous independent variables. Effects of interactions between time, matric potential and NH_4^+-N were also tested, as they were physiologically meaningful. For the pure culture experiment, the effects of the osmo-inducer nature (sorbitol vs. NaCl) and osmotic potential on each strain growth rate was assessed by two-way ANOVA after $\log_{10}$ transformation of growth rate data. Tukey HSD multiple *post-hoc* tests were used to identify differences among the treatment means detected by ANOVAs. All models and results (including effect size) are shown in Supplementary Information.

3. Results

3.1. Influence of matric potential and NH_4^+ amendment on AOA and AOB abundance and nitrification activity

Pre-treatment of soil microcosms successfully reduced soil inorganic NH_4^+, enabling measurement of nitrification, and led to a small decrease in pH to 6.1 ± 0.11 immediately prior to application of NH_4^+ and matric potential treatments (Fig. S1). Ammonia oxidiser activity during incubation was assessed through changes in NH_4^+ and NO_3^- concentrations. In some cases, NO_3^- concentration increased linearly, enabling calculation of ammonia oxidation rate, but this was not possible for all treatments and ammonia oxidation activities were therefore compared by ANOVA of NH_4^+ and NO_3^- concentrations during incubation for 28 days.

Overall, initial NH_4^+, matric potential and their interaction significantly influenced nitrification (Fig. 1). Ammonia oxidising activity was greatest at high matric potential (-0.019 MPa) (i.e. low water stress), where oxidation of high initial NH_4^+ (60 μg NH_4^+-N g^{-1}) was almost complete by day 28 (Fig. 1A), and NH_4^+ concentration was negligible (≤1 μM) by day 7 for both low (0.6 μg NH_4^+-N g^{-1}) and medium (6 μg NH_4^+-N g^{-1}) initial NH_4^+ concentrations

(Figs. 1B, C). Final NO_3^- concentration increased with increasing initial NH_4^+ concentration (Fig. 1D - F) from an initial concentration of ~11 μg NO_3^--N g^{-1} and changes in NH_4^+ and NO_3^- concentrations were stoichiometrically equivalent. However, final NO_3^- concentration was always greater than the concentration of added NH_4^+, due to additional supply through mineralisation of native soil organic nitrogen, equivalent, respectively, to 2.5, 0.98 and 0.93 μg NH_4^+-N g^{-1} at high, medium and low matric potentials after incubation for 28 days. Ammonia oxidation activity was lower at medium and low matric potentials (Fig. 1B and C) than at high matric potential (Fig. 1A). At medium matric potential, final NO_3^- concentration was greatest at high initial NH_4^+ concentration but did not differ significantly between medium and low initial NH_4^+ (Fig. 1E). At low matric potential (highest water stress), there was little ammonia oxidation activity (Fig. 1C) and NO_3^- production was low and not influenced by initial NH_4^+ concentration (Fig. 1F) As expected, ammonia oxidation led to a decrease in soil pH, which was statistically significant only in the soil with the highest matric potential and initial ammonium concentration (Fig. S1).

AOA *amoA* abundance was higher than that of AOB *amoA* throughout incubation of all microcosms, irrespective of matric potential and initial NH_4^+ concentration (Fig. 2). Three-way ANOVAs and *post-hoc* Tukey tests identified different responses of AOA and AOB to microcosm treatments. Moreover, the effects of measured soil NH_4^+-N concentration and pH were further explored using generalised linear models (Supplementary Information). Both AOA and AOB abundances varied significantly with pH (p=0.035) and decreased with matric potential (p<0.013), but AOA abundance was not significantly correlated with soil NH_4^+ concentration (p=0.841), while temporal changes in AOB abundance correlated strongly with NH_4^+ concentration (p=0.009). While AOA increased in abundance significantly at high matric potential, there was no significant AOA growth, and even a significant decrease in AOA *amoA* abundance, at medium and low matric potentials (Fig. 2A - C, $p<10^{-7}$). Overall, both high matric potential (p=0.001) (low water stress) and high NH_4^+ concentration (p=0.014) increased

AOB abundance after incubation for 28 days (Fig. 2D - F). AOB did not grow at medium and low matric potentials but abundance increased significantly following incubation at high matric potential with high initial NH_4^+ concentration (p=0.039).

At the end of the 28-day incubation period, the AOA:AOB *amoA* ratio was significantly lower (p=0.007) at low (4.30±0.27) and medium (4.46±0.38) matric potentials, where water stress was greatest, than at high matric potential (7.5±1.2). This ratio was also significantly lower at high NH_4^+ (3.80±0.19) than at medium (6.60±1.21) and low (5.91±1.93) NH_4^+ concentrations (p=0.008).

3.2. Influence of osmotic potential on growth of AOA and AOB in liquid batch culture

The influence of osmotic potential on AO growth was investigated by measuring the effect of two osmo-inducers, NaCl and sorbitol, on growth of two AOA and two AOB (Fig. 3). Because of differences in culture medium composition between strains, similar concentrations of NaCl or sorbitol led to different osmotic potentials and a greater osmotic potential range for AOA than AOB (Table S1). All strains were more sensitive to the effects of NaCl than sorbitol, at equivalent osmotic potentials, presumably due to direct toxicity of NaCl as shown by ANOVAs ($p < 10^{-12}$; Fig 3, Supplementary Iinformation). Maximum specific growth rates of both AOA and AOB decreased with increasing NaCl concentration and AOA (Fig. 3C and D) were more sensitive than AOB (Fig. 3A and B). AOA growth was inhibited following even a slight decrease in osmotic potential (Fig. 3C and D, $p < 10^{-4}$). Within AOA, *Ca.* N. franklandus was less sensitive than *Ca.* N. sinensis, whose growth was completely inhibited by 0.1 M NaCl. Inhibition of growth by sorbitol was greater for both AOA than for *N. europaea*, with complete inhibition of *Ca.* N. sinensis at -67 MPa (Fig. 3B) and a 3-fold reduction in maximum specific growth rate of *Ca.* N. franklandus at -98 MPa (Fig. 3B). *N. europaea* was inhibited by sorbitol (Fig. 3A) but maximum specific growth rate of the AOB *N. multiformis* increased with increasing sorbitol concentration (Fig. 3B).

4. Discussion

Climate change is predicted to involve more frequent and drastic drought events (Kovats et al., 2014). Understanding the impacts of such events on soil biogeochemical processes, including nitrification, and its influence on N availability for plant growth and nitrous oxide emissions, is critical for appropriate prediction and mitigation of the consequences of climate change (Gruber and Galloway, 2008). Previous studies have demonstrated effects of drought on nitrification rate and ammonia oxidiser community structure and activity (Stark and Firestone, 1995; Placella and Firestone, 2013; Fuchslueger et al., 2014), and AOA:AOB abundance ratio has been found to decrease during drought and following rewetting (Gleeson et al., 2010; Thion and Prosser, 2014). This decrease was proposed to arise from different responses of AOA and AOB to drought-induced changes in NH_4^+ concentration (Thion and Prosser, 2014), but may alternatively result from different responses of AOA and AOB to water stress. Because bacteria and archaea appear to possess comparable mechanisms of adaptation to water stress (Roeßler and Müller, 2001), although using different compatible solutes, there is no *a priori* reason to expect differences between AOA and AOB.

This study aimed to challenge the hypothesis that changes in AOA and AOB relative activities were due to drought-induced changes in NH_4^+ availability, testing the alternative hypothesis that differential activity changes resulted from differences in sensitivity to water stress. This was achieved using two complementary approaches, in soil microcosms and in pure AO cultures. The effects of water stress and NH_4^+ concentration on soil AO were distinguished through manipulation of matric potential of soil microcosms amended with different concentrations of NH_4^+. At high matric potential, AOB growth occurred in proportion to initial ammonium concentration; AOA also grew, but growth was greatest at the intermediate NH_4^+ concentration. This resulted in lower AOA:AOB ratio with high NH_4^+ amendment, consistent with previous reports that AOB, rather than AOA, are favoured by supply of inorganic NH_4^+ at

high concentration, equivalent to those frequently found following inorganic nitrogen fertilisation (Verhamme et al., 2011; Hink et al., 2017b, 2018). AO growth was reduced in microcosms in which matric potential was reduced, regardless of NH_4^+ concentration. At the medium matric potential, NH_3 oxidation was significantly reduced, AOB abundance did not increase significantly and AOA abundance decreased, suggesting AOA cellular death. At the lowest matric potential, no activity was detected and, again, AOB survived while AOA probably died. Consequently, AOA:AOB ratio decreased with matric potential.

The reduction in ammonia oxidiser activity with increased water stress therefore resulted in differential effects on AOA and AOB abundances that were independent of NH_4^+ concentration, suggesting that matric potential, and not NH_4^+ concentration, may be the more important factor influencing AO activities and greater sensitivity of AOA to drought. Water stress can result from matric and osmotic stresses and greater sensitivity of AOA to the latter was further supported by determining growth rates of pure cultures of AOA and AOB in the presence of the osmo-inducers NaCl and sorbitol. *N. multiformis* is typical of soil AOB. *N. europaea*, originally isolated from soil, is considered to be less important in terrestrial environments but has been the subject of most physiological studies of AOB, including studies of osmotic stress. The two AOA were also isolated from soil, *Ca.* N. franklandus from neutral soil and *Ca.* N. sinensis Nd2 from acid soil. All strains were inhibited by increasing osmotic stress using NaCl, which possibly resulted in part from cytotoxicity of NaCl itself, rather than osmotic stress. Previous studies have reported similar effects of NaCl on the AOB *N. europaea, N. eutropha, Nitrosomonas oligotropha* and *Nitrosococcus mobilis* (Wood and Sörensen, 1998; Claros et al., 2010; Koops et al., 1976) and the AOA *Ca.* Nitrosotenuis cloacae (Li et al., 2016). However, sorbitol is unlikely to be imported into AOA or AOB cells due to its relatively large molecular weight (182 g mol^{-1}) and there is, to our knowledge, no evidence for bacterial or archaeal sorbitol cytotoxicity. Sorbitol is a proven and efficient osmo-inducer (Suga et al., 2003) and it is likely that effects on AO were due to changes in osmotic stress, rather than

sorbitol toxicity. The reduced effect observed on the AOB *N. multiformis* may be due to its potential to metabolise sorbitol (Norton et al., 2008). Increasing osmotic stress through increased sorbitol concentration completely inhibited growth of the AOA *Ca.* N. sinensis and inhibition of *Ca.* N. franklandus was greater than that of the AOB *N. multiformis* and *N. europaea* over the range investigated. While caution must be exercised in generalising findings from this limited number of laboratory isolates to natural communities of AOA and AOB, these data do suggest greater sensitivity of AOA to osmotic stress.

In conclusion, our data provide the first evidence for differences in sensitivity of soil AOA and AOB to the combined effects of water stress and increased matric and osmotic potentials. The mechanisms leading to these differences remain unknown but physiological studies of a limited number of strains demonstrated greater sensitivity of AOA to osmotic stress. The different characteristics of AOA and AOB, in particular differences in nitrous oxide emissions (Hink et al., 2017b) and responses to soil pH (Nicol and Prosser, 2012) and fertilisation strategies (Verhamme et al., 2011; Hink et al., 2018), coupled with greater AOA sensitivity to drought, therefore increase our understanding of the consequences of drought on this important biogeochemical cycling process and on the consequences for nitrogen fertiliser use efficiency and climate change.

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Appendix A. Supplementary Information

Supplementary data related to this article can be found at ..

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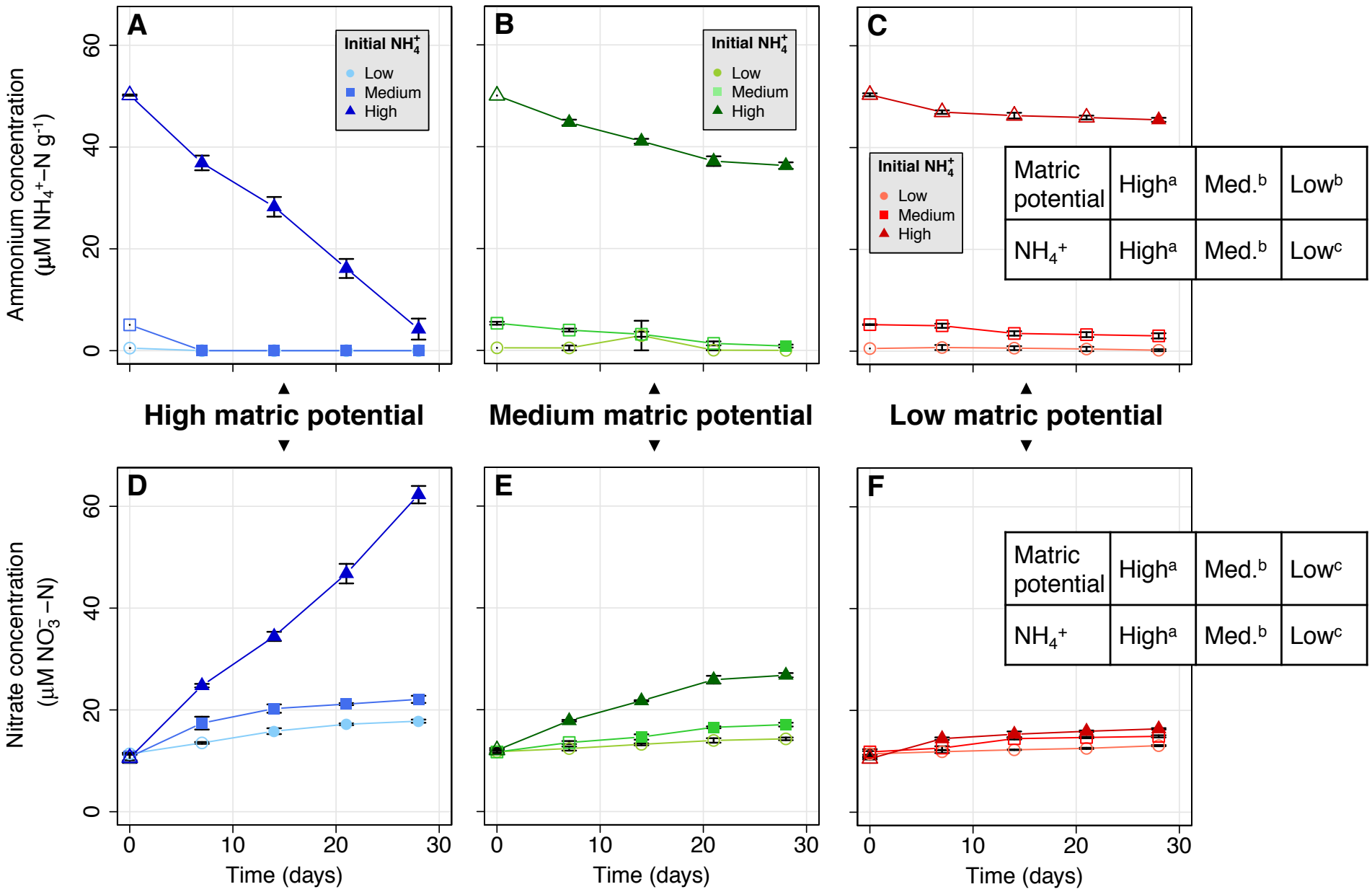
Figure legends

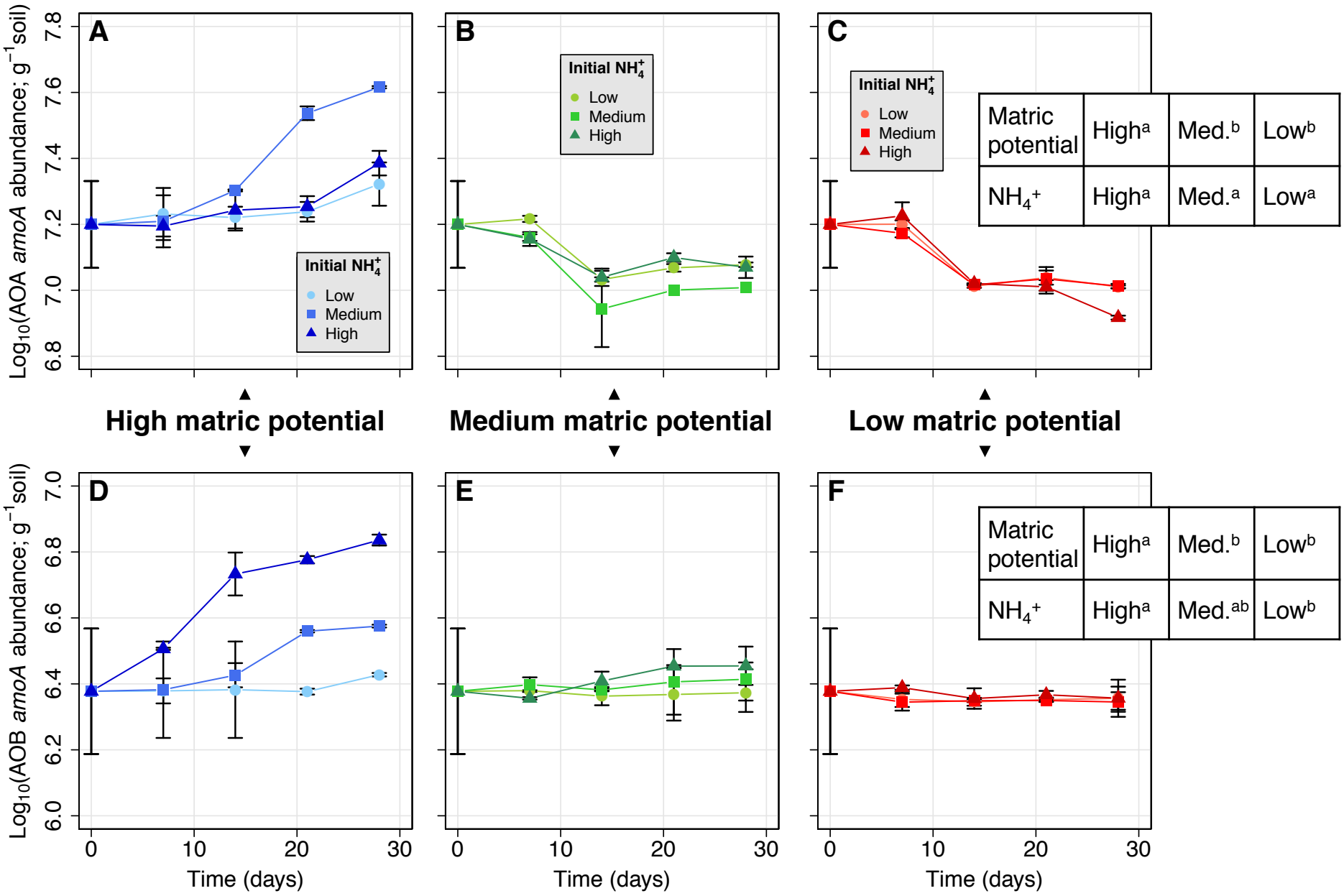
Figure 1. Changes in NH_4^+-N (A - C) and NO_3^--N concentrations (D - F) during incubation of soil microcosms for 28 days at (A) high (-0.019 MPa), (B) medium (-0.051 MPa) and (C) low (-0.08 MPa) matric potential (low, medium and high water stress, respectively) and with initial high (60 μg NH_4^+-N g^{-1}, triangle), medium (6 μg NH_4^+-N g^{-1}, square) and low (0.6 μg NH_4^+-N g^{-1}, circle) NH_4^+-N concentration. Filled symbols indicate significant differences between each measured concentration and the initial concentration for the same treatment as tested by 3-way ANOVA followed by *post-hoc* Tukey test. Embedded tables show Tukey test grouping following 3-way ANOVAs with different letters indicating significant difference between levels of MP or initial NH_4^+-N concentration. Data are presented as mean and standard error of samples from triplicate microcosms.

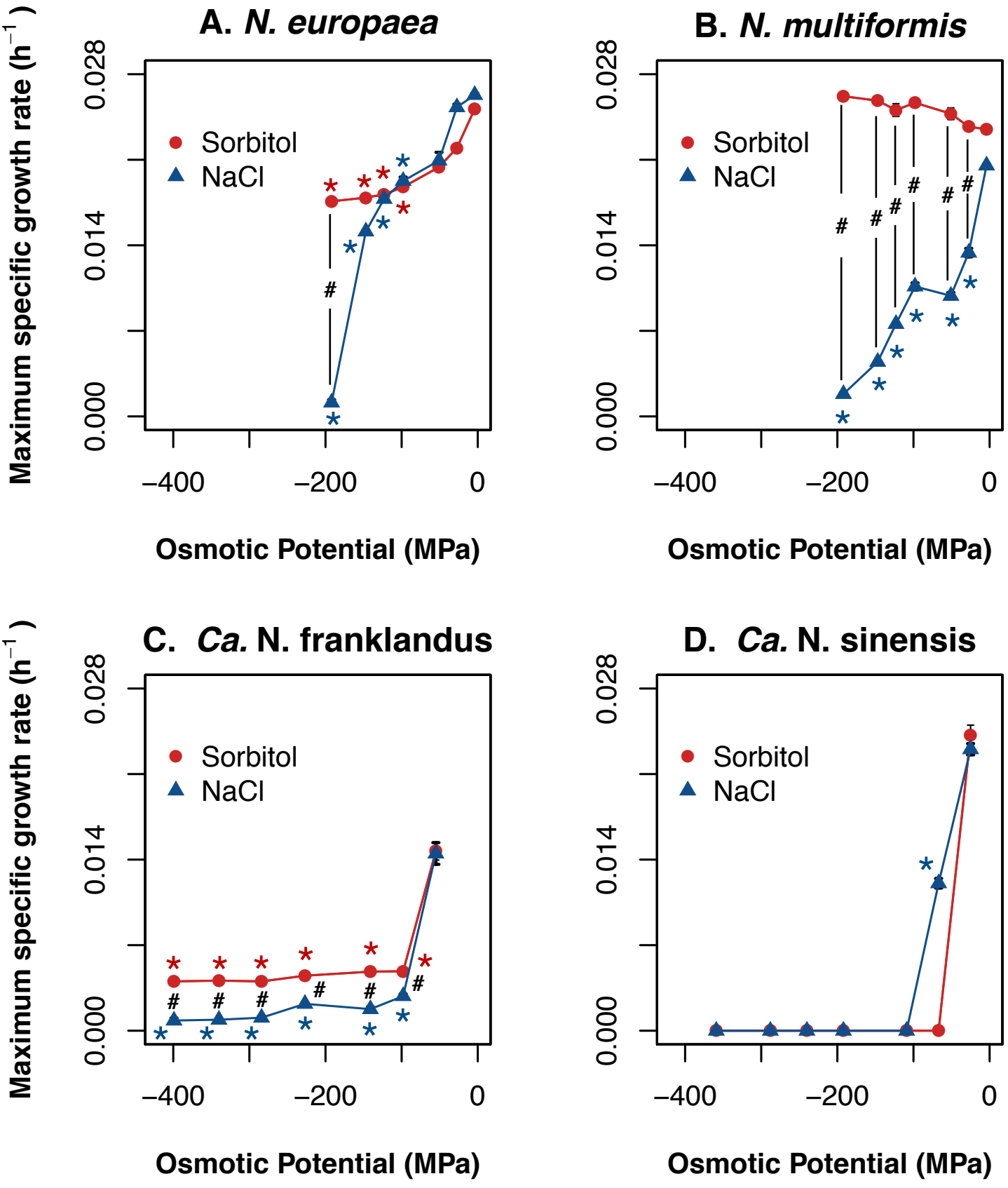
Figure 2. Changes in AOA (A - C) and AOB (D - F) *amoA* abundances during incubation of soil microcosms for 28 days at (over 28-day incubation of soil microcosms at (A) high (-0.019 MPa), (B) medium (-0.051 MPa) and (C) low (-0.08 MPa) matric potential and with initial high (60 μg NH_4^+-N g^{-1}, triangle), medium (6 μg NH_4^+-N g^{-1}, square) and low (0.6 μg NH_4^+-N g^{-1}, circle) NH_4^+-N concentration. Embedded tables show Tukey test grouping following 3-way ANOVAs with different letters indicating significant difference between levels of MP or initial NH_4^+-N concentration. Data are presented as mean and standard error of samples from triplicate microcosms.

Figure 3. Maximum specific growth rate of model strains of AOB (A. *N. europaea* and B. *N. multiformis*) and AOA (C. *Ca.* N. franklandus and D. *Ca.* N. sinensis) in liquid batch culture as a function of osmotic potential, using NaCl (blue) or sorbitol (red) as osmo-inducer. Data

are presented as mean and standard error of growth rates determined from triplicate cultures. Symbols indicate significant differences as revealed by two-way (osmo-inducer nature and osmotic potential) ANOVAs followed by Tukey *post-hoc* tests. For clarity, only differences between control cultures (without osmo-inducer) and cultures with either sorbitol (red stars) or NaCl (blue stars) and differences between the two osmo-inducers applied at the same concentrations, resulting in the same osmotic potential (black hash) are shown. This could not be calculated for *Ca*. N. sinensis (panel D), whose growth was not detectable with sorbitol or NaCl at concentrations higher than 0.05 M.


A
B
C
D
E
F
Initial NH_4^+
Low
Medium
High
Ammonium concentration
(µM NH_4^+–N g^{-1})
Nitrate concentration
(µM NO_3^-–N)
0
20
40
60
0
10
20
30
Time (days)
High matric potential
Medium matric potential
Low matric potential
Matric potential | High[a] | Med.[b] | Low[b]
NH_4^+ | High[a] | Med.[b] | Low[c]
Matric potential | High[a] | Med.[b] | Low[c]
NH_4^+ | High[a] | Med.[b] | Low[c]


A
B
C
D
E
F
Log10(AOA amoA abundance; g−1soil)
Log10(AOB amoA abundance; g−1soil)
Initial NH4+
Low
Medium
High
Matric potential | High[a] | Med.[b] | Low[b]
NH4+ | High[a] | Med.[a] | Low[a]
Matric potential | High[a] | Med.[b] | Low[b]
NH4+ | High[a] | Med.[ab] | Low[b]
High matric potential
Medium matric potential
Low matric potential
Time (days)


A. N. europaea
B. N. multiformis
C. Ca. N. franklandus
D. Ca. N. sinensis
Maximum specific growth rate (h^{-1})
0.000
0.014
0.028
Sorbitol
NaCl
–400
–200
0
Osmotic Potential (MPa)

Differential sensitivity of ammonia oxidising archaea and bacteria to matric and osmotic potential

Supplementary information

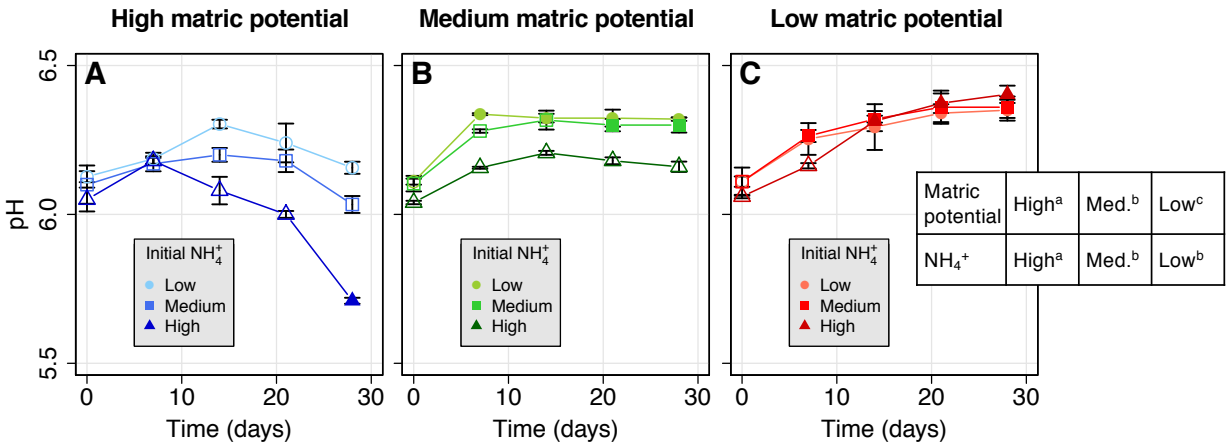
Table S1. Osmotic potential of culture media adjusted with NaCl and sorbitol. Osmotic potential (ψ) of the culture media was calculated taking into account differences in medium composition, using equation $\psi = - M\ i\ R\ T$ (Lewis, 1908), where M is the molar concentration (mol l^{-1}) of the solute, i is the van't Hoff factor of the medium, R is the ideal gas constant and T is the absolute temperature (^{o}K) (Lewis, 1908).

Osmo-inducer concentration (mol l^{-1})	Osmotic potential (MPa)		
	AOB	*Ca.* N. franklandus	*Ca.* N. sinensis nd2
0.00	-4.00	-55.0	-25.0
0.05	-27.4	-98.0	-67.0
0.10	-50.9	-141	-109
0.20	-98.0	-227	-192
0.25	-123	-284	-240
0.30	-147	-340	-288
0.40	-192	-399	-359

References

Lewis, G. N., 1908). The osmotic pressure of concentrated solutions, and the laws of the perfect solution. Journal of the American Chemical Society, 30, 668-683.

Supplementary Figure 1. Changes in pH during incubation of soil microcosms for 28 days at (A) high (-0.019 MPa), (B) medium (-0.051 MPa) and (C) low (-0.08 MPa) matric potential and with initial high (50 mM, triangle), medium (5 mM, square) and low (0.5 mM, circle) NH_4^+-N concentration. Filled symbols indicate significant differences between each pH and initial pH of the corresponding treatment, tested by 3-way ANOVA followed by *post-hoc* Tukey test. Embedded tables show Tukey test grouping following ANOVAs with different letters indicating significant difference between levels of MP or initial NH_4^+-N concentration. Data are presented as mean and standard error of samples from triplicate microcosms.



Matric potential	High[a]	Med.[b]	Low[c]
NH_4^+	High[a]	Med.[b]	Low[b]

Statistical models and results

Tables below show statistics calculated by 3-way ANOVAs testing the effect of independent categorical variables: ammonium amendment (NH_4^+): High, Medium or Low; MP: High, Medium or Low; Time: T_0, T_7, T_{14}, T_{21}, T_{28}) and generalised linear models testing regressions between AOA or AOB abundance and MP and Time as categorical variables and measured NH_4^+-N concentration ($[NH_4^+]$) and pH as continuous variables, with:

- *Df*: degree of freedom
- *SS*: sum of squared
- *MS*: mean square
- Ω^2: effect size, where 0 indicates no effect and ±1 indicates maximum effect (where 100% of the variance of the dependent variable is explained by the independent variable)
- *SE*: standard error or estimated intercept or slope
- *Min*, *1st Q*, *Med*, *3rd Q* and *Max*: minimum, 1st quartile, median, 3rd quartile and maximum residual value, respectively.
- *AIC*: Akaike information criterion

Lines shaded in orange highlight significant effects.

1 Soil microcosms

1a Soil NH_4^+ concentration

Model:

*aov($[NH_4^+]$ ~ NH_4^+ * MP * Time)*

Output:

Effect	Df	SS	MS	F-value	p-value	Ω^2
NH_4^+	2	112.8	56.38	11501.1	**4×10^{-109}**	**0.84**
MP	2	4.0	1.98	403.9	**1×10^{-45}**	**0.03**
Time	4	3.3	0.83	169.8	**5×10^{-41}**	**0.02**
NH_4^+ x MP	4	4.9	1.22	249.8	**8×10^{-48}**	**0.04**
NH_4^+ x Time	8	3.8	0.48	98.0	**5×10^{-41}**	**0.03**
MP x Time	8	1.7	0.20	42.3	**3×10^{-27}**	**0.01**
NH_4^+ x MP x Time	16	3.1	0.19	39.6	**1×10^{-33}**	**0.02**
Residuals	90	0.4	0.01			0.00

1b Soil NO_3^- concentration

Model:

*aov($[NO_3^-]$ ~ NH_4^+ * MP * Time)*

Output:

Effect	Df	SS	MS	F-value	p-value	Ω^2
NH_4^+	2	28.7	14.4	1236.0	**4×10^{-66}**	**0.21**
MP	2	24.0	12.0	1035.9	**7×10^{-63}**	**0.18**
Time	4	23.4	5.9	503.7	**1×10^{-60}**	**0.17**
NH_4^+ x MP	4	17.7	4.4	381.4	**2×10^{-55}**	**0.13**
NH_4^+ x Time	8	14.4	1.8	154.9	**4×10^{-49}**	**0.11**
MP x Time	8	12.4	1.5	133.5	**2×10^{-46}**	**0.09**
NH_4^+ x MP x Time	16	12.1	0.8	65.0	**3×10^{-42}**	**0.09**
Residuals	90	1.0	0.0			0.00

1c AOA abundance

ANOVA

Model:

*aov(AOA ~ NH_4^+ * MP * Time)*

Output:

Effect	Df	SS	MS	F-value	p-value	Ω^2
NH_4^+	2	0.6	0.32	0.7	0.503	-0.002
MP	2	41.4	20.73	44.01	**5×10^{-14}**	**0.301**
Time	4	7.2	1.82	3.87	**0.006**	**0.040**
NH_4^+ x MP	4	5.8	1.46	3.10	**0.019**	**0.029**
NH_4^+ x Time	8	2.1	0.26	0.56	0.805	-0.012
MP x Time	8	28.9	3.61	7.68	**9×10^{-8}**	**0.187**
NH_4^+ x MP x Time	16	5.2	0.32	0.69	0.792	-0.017
Residuals	90	42.4	0.471			0.00

Generalised linear model

Model:

*glm(AOA ~ MP * [NH_4^+] * Time + pH)*

- No biologically meaningful effect of interactions between pH and the other factors
- No known random factor
- Residuals were assumed to have normal distribution

Output:

- Residuals: Shapiro-Wilk's test confirms normality (p = 0.0014)

Min	1st Q	Med	3rd Q	Max
-2.03	-0.31	0.00	0.30	1.46

- Coefficient: Intercept

	Estimate	SE	t-value	p-value
(Intercept)	*0.098*	*0.184*	*0.535*	*0.593*

- Coefficient: Slopes

	Estimate	SE	t-value	p-value
MP Low	0.084	0.261	0.320	0.749
MP Medium	-0.011	0.259	-0.044	0.965
$[NH_4+]$	0.035	0.174	0.201	0.841
Time	0.025	0.015	1.647	0.102
pH	**-0.196**	**0.092**	**-2.129**	**0.035**
MP Low x $[NH_4^+]$	0.006	0.233	0.026	0.979
MP Medium x $[NH_4^+]$	-0.111	0.237	-0.470	0.639
MP Low x Time	**-0.061**	**0.021**	**-2.944**	**0.004**
MP Medium x Time	**-0.053**	**0.019**	**-2.750**	**0.007**
$[NH_4^+]$ x Time	-0.034	0.018	-1.868	0.064
MP Low x $[NH_4^+]$ x Time	0.028	0.021	1.353	0.179
MP Medium x $[NH_4^+]$ x Time	0.038	0.021	1.794	0.075

- AIC : 299.46

1d AOB abundance

ANOVA

Model:

*aov(AOB ~ NH_4^+ * MP * Time)*

Output:

Effect	Df	SS	MS	F-value	p-value	Ω^2
NH_4^+	2	8.0	4.04	4.50	**0.014**	**0.05**
MP	2	15.7	7.85	8.74	**4×10^{-4}**	**0.10**
Time	4	4.4	1.11	1.24	0.298	**0.01**

NH_4^+ x MP	4	9.4	2.36	2.63	**0.039**	**0.04**
NH_4^+ x Time	8	3.5	0.44	0.49	0.859	-0.03
MP x Time	8	7.4	0.93	1.04	0.411	**0.01**
NH_4^+ x MP x Time	16	4.3	0.27	0.30	0.996	**-0.08**
Residuals	90	80.8	0.89			0.00

Generalised linear model

Model:

*glm(AOB ~ MP * [NH_4^+] * Time + pH)*

- No biologically meaningful effect of interactions between pH and the other factors
- No known random factor
- Residuals were assumed to have normal distribution

Output:

- Residuals: Shapiro-Wilk's test confirms normality (p = 1.6 x 10^{-7})

Min	1st Q	Med	3rd Q	Max
-1.48	-0.32	0.02	0.27	2.34

- Coefficient: Intercept

	Estimate	SE	t-value	p-value
(Intercept)	*-0.513*	*0.218*	*-2.348*	*0.020*

- Coefficient: Slopes

	Estimate	SE	t-value	p-value
MP Low	0.060	0.309	0.193	0.847
MP Medium	0.155	0.307	0.505	0.614
[NH_4+]	-0.108	0.207	-0.523	0.602
Time	**0.084**	**0.018**	**4.729**	**7 x 10^{-6}**
pH	**-0.383**	**0.108**	**-3.510**	**0.001**
MP Low x [NH_4^+]	0.063	0.276	0.227	0.821
MP Medium x [NH_4^+]	-0.059	0.280	-0.211	0.833
MP Low x Time	**-0.062**	**0.025**	**-2.514**	**0.013**
MP Medium x Time	**-0.063**	**0.023**	**-2.737**	**0.007**

[NH_4^+] x Time	**0.074**	**0.022**	**3.397**	**0.001**
MP Low x [NH_4^+] x Time	**-0.070**	**0.025**	**-2.818**	**0.006**
MP Medium x [NH_4^+] x Time	**-0.069**	**0.025**	**-2.742**	**0.007**

- AIC : 344.89

1e Soil pH

Model:

*aov(pH ~ NH_4^+ * MP * Time)*

Output:

Effect	Df	SS	MS	F-value	p-value	Ω^2
NH_4^+	2	16.6	8.3	55.1	**3×10^{-16}**	**0.12**
MP	2	31.2	15.6	103.6	**5×10^{-24}**	**0.23**
Time	4	27.1	6.8	45.1	**1×10^{-20}**	**0.20**
NH_4^+ x MP	4	7.7	1.9	12.7	**3×10^{-8}**	**0.05**
NH_4^+ x Time	8	2.0	0.2	1.7	0.121	0.01
MP x Time	8	25.9	3.2	21.6	**6×10^{-18}**	**0.18**
NH_4^+ x MP x Time	16	10.0	0.6	4.2	**7×10^{-6}**	**0.06**
Residuals	90	13.5	0.2	NA		0.00

2 Pure culture experiment

Tables below show statistics calculated by 2-way ANOVAs testing the effect of osmo-inducer nature (sorbitol or NaCl) and osmotic potential (OP) on specific growth rate (GR) on AOA and AOB strains.

2a. N. europaea

Model:

*aov(Log_{10}GR ~ osmo-inducer * OP)*

Output:

Effect	Df	SS	MS	F-value	p-value	Ω^2
Osmo-inducer	1	1.6	1.61	141	**2×10^{-12}**	**0.06**
OP	6	13.0	2.17	190	**2×10^{-21}**	**0.52**

Osmo-inducer x OP	6	10.1	1.69	148	**7×10^{-20}**	**0.40**
Residuals	28	0.3	0.01			0.00

I. 2b N. multiformis

Model:

*aov(Log$_{10}$GR ~ osmo-inducer * OP)*

Output:

Effect	Df	SS	MS	F-value	p-value	Ω^2
Osmo-inducer	1	14.15	14.15	7719	**1×10^{-35}**	**0.55**
OP	6	5.21	0.87	474	**8×10^{-27}**	**0.20**
Osmo-inducer x OP	6	6.23	1.04	566	**7×10^{-28}**	**0.24**
Residuals	28	0.05	0.01			0.00

II *2c* Ca. *N. franklandus*

Model:

*aov(Log$_{10}$GR ~ osmo-inducer * OP)*

Output:

Effect	Df	SS	MS	F-value	p-value	Ω^2
Osmo-inducer	1	9.8	9.84	345	**2×10^{-17}**	**0.30**
OP	6	18.8	3.14	110	**3×10^{-18}**	**0.57**
Osmo-inducer x OP	6	3.1	0.51	18	**2×10^{-8}**	**0.09**
Residuals	28	0.8	0.03			0.00

III *2d* Ca. *N. sinensis*

Model:

aov(Log$_{10}$GR ~ OP)

NB: only the significance of the effect of NaCl, at only the lowest concentration, could be tested for Ca. N. sinensis, because the strain did not grow in any of the cultures amended with sorbitol or NaCl at concentrations higher than 0.05 M.

Output:

Effect	Df	SS	MS	F-value	p-value	Ω^2
OP	1	0.63	0.63	280	**7×10^{-5}**	**0.98**
Residuals	4	0.01	0.01			0.00